INFERRING PROPERTIES OF ANCIENT CYANOBACTERIA FROM BIOGEOCHEMICAL ACTIVITY AND GENOMES OF SIDEROPHILIC CYANOBACTERIA. I.I. Brown[1], S. G. Tringe[2], K.L. Thomas-Keprta[1], D.A. Bryant[3], S.A. Sarkisova[1], K. Malley[4], O. Sosa[4], C. G. Klatt[5], D.H. Garrison[1] and D.S. Mckay[6]. [1]SARD/JSC, Mail Code: JE 23, ESCG, P. O. Box 58447, Houston, TX,. [2]DOE Joint Genome Institute, 2800 Mitchell Drive, Bldg 400, Walnut Creek, CA 94596. [3]The Pennsylvania State University, [4] NASA/USRP, [5]Montana State University. [6]NASA JSC.

Introduction: Verifying the links between genomic features in living organisms and the mineralized signatures generated by these organisms will help to reveal traces of life on Earth and beyond.

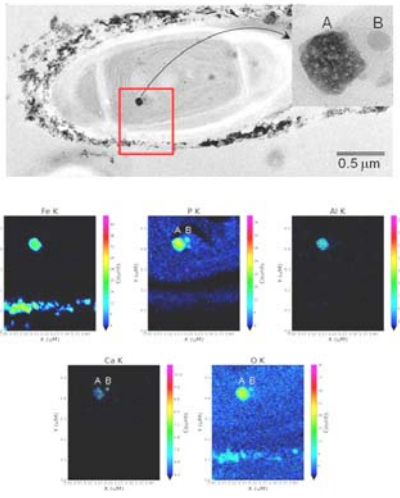
Among contemporary environments, iron-depositing hot springs (IDHS) may represent one of the most appropriate natural models [1] for insights into ancient life since organisms may have originated on Earth and possibly Mars in association with hydrothermal activity and high [Fe^{2+}] [2-6]. Siderophilic or "iron-loving" cyanobacteria (CB) inhabiting IDHS may have genomic features and properties similar to those of ancient organisms because abundant Fe^{2+} in IDHS has a strong potential to increase the magnitude of oxidative stress [7]. It is known that the mechanism of Fe homeostatsis in non-siderophilic CB is mainly based on Fe^{2+} intracellular mineralization within bacterioferritin and bacterioferritin-like proteins [8,9]. However, this mechanism can be insufficient to maintain Fe homeostasis in siderophilic CB because [Fe^{2+}] in IDHS is about 77 µm, ~700 times higher than in other water bodies [1]. That is why specific and/or additional proteins for Fe mineralization by siderophilic CB are expected. Inorganic polyphosphates (PPi) are known to increase the viability of prokaryotes under heavy metal concentrations and UV stress conditions [10, 11]. PPi have also been proposed as biosignatures [9]. However, the molecular mechanisms of the maintenance of Fe homeostasis in siderophilic CB and the role of PPi for this process is poorly understood.

Here we present preliminary results describing a new mechanism of Fe mineralization in siderophilic CB, the effect of Fe on the generation of PPi bodies in CB, and preliminary analysis of the diversity of proteins involved in the prevention of oxidative stress in phototrophs inhabiting IDHS.

Material and methods: CB and environmental DNA studied were isolated from IDHS located in the Greater Yellowstone Area (USA). Genomic and metagenomic results were obtained by standard methods. The Fe mineralization process by CB cultures was also studied *in vitro*. Thin sections were analyzed using a JEOL 2000 FX and JEOL 2500 SE TEM at 200 keV. Both are equipped with light element EDX detectors.

Results: *Formation of Fe-Oxides*. The cultivation of three siderophilic CB with 0.6 mM Fe^{3+} led to the formation of extracellular Fe-Oxide nanoparticles (typically < 50 nm in size) and intracellular Fe-rich particles (Fig. 1). The non-siderophilic CB *Synechocystis* sp. PCC 6803 neither accumulated bulk Fe precipitate on the cellular sheath nor generated intracellular Fe-rich particles. In a medium with low [P], which is typical in IDHS such as Chocolate Pots (CP) Hot Spring in Yellowstone National Park, the external Fe-oxides have a texture (fine grain/amorphous to poorly crystalline) and composition (Fe, O) consistent with ferrihydrite Fig. 1). Intracellular Fe-rich particles are also amorphous but contain additional elements including P, Al and Ca (Fig.1). The non-siderophilic CB *Synechocystis* sp. PCC 6803 neither accumulated Fe precipitates on the cellular sheath nor generated intracellular Fe-rich particles.

Fig. 1. Extra- and intracellular Fe-oxide particles associated with siderophilic CB JSC-1**.** *Top*: TEM view of a single cell encased in electron dense Fe-Oxides. Two intracellular components are highlighted in the ROI (red box): (A) intracellular Fe-oxide; and, (B) a spatially related internal body. *Lower*: Element maps for Fe, P, Al, Ca, O of the ROI showing the extracellular Fe-oxides are essentially free of P while the internal Fe-oxide (A) contains P, Al, and Ca and the associated body (B) is composed of P, Ca, and O.



We suggest the Fe-rich particles located within the cells are biogenic in nature and that those Fe-oxides located on the external sheaths have an inorganic origin. However, they could be subjected to further modification by microbial cells, *e.g.* P leaching.

Metagenome studies. Comparative analysis of the genes encoding enzymes predicted to maintain intra-

cellular Fe homeostasis in organisms from an IDHS and two non-Fe depositing hot springs. Putative Fe homeostasis genes from CB were most abundant in the CP bacterial community. In contrast, the number of Fe homeostasis genes from CB in Octopus (OS) and Mushroom (MS) springs, where the Fe concentrations are 300 to 700 times lower, was less than the number of anoxygenic phototrophs.

Synechococcus sp. JA-2-3B'a (2-13), which has been sequenced recently [10], was shown to be dominant CB in a mat collected from CP. This species considered as one of the deeply rooted organisms in CB phylogeny [11]. Another relatively deeply rooted CB, clone SM1F09 (acces. # AF445691), was also found in this mat. Thus, one may speculate that molecular features of deeply rooted species of siderophilic CB help them withstand very high [Fe^{2+}] and the resulting oxidative stress [7]. These properties helped to make them a dominant organisms in IDHS and probably in the late Archean ocean.

The sequences of proteins (Fig. 2) such as DNA-binding ferritin-like protein (**Dps**), polyphosphate kinase (**PPK**) and ferric ABC transporter permease (**FeT**) from *Roseiflexus* sp. RS-1 have similar identity to sequences in metagenomes from CP and MS but not for OS. **FeT** and **PPK** found in the genome of *Synechococcus* sp. JA-2-3B'a (2-13) gave very high identities to CP and MS metagenomes but were not found in the OS metagenome. However, the identity of bacterioferritin comigratory protein (**Bcp**) in *Synechococcus* sp. JA-2-3B'a(2-13) genome was close to 100% amino acid identity in CP and MS metagenomes but below 60% in OS metagenome. Proteins with similar roles in Fe homeostasis from 5 non-siderophilic CB displayed less than 50% identity to all metagenomes studied (not shown). Surprisingly, ferrous iron transport protein B (**FeoB**) from *Synechococcus* sp. PCC 6803 displays substantial identity to sequences in CP and MS metagenomes (Fig.2). These observations suggest that physico-chemical parameters in each spring studied render specific sets of proteins to maintain Fe homeostasis in organisms inhabiting those springs.

PPi analysis. Fe stimulated the generation of PPi bodies in JSC-1 when the medium had low [P] (0.04 mM) (Fig.3) and preserved PPBs degradation in JSC-1 cells transferred to a medium without added P (not shown). Iron did not seem to affect the number of PPBs in cells of *Synechocystis* sp. PCC 6803. The different reactions of siderophilic and non-siderophilic CB could be explained by the fact that Synechocystis sp. 6803 has only one type of PPK while the JSC-1 genome has 4 paralogous ORFs predicted to encode this enzyme (not shown). These observations suggest that fossils of ancient siderophilic CB, which existed before the Great Oxidation Event, [12] might be richer with ferric phosphates than their descendants inhabiting Fe^{2+} poor water bodies.

Fig. 2. Percent of identity of proteins involved in the maintenance of Fe homeostasis in organisms found in IDHS to metagenomes of CB mats in Chocolate Pots, Mushroom and Octopus hot springs**.** Proteins with identity above 50% amino acid identity were used for the analysis. Abbreviations are explained in the text.

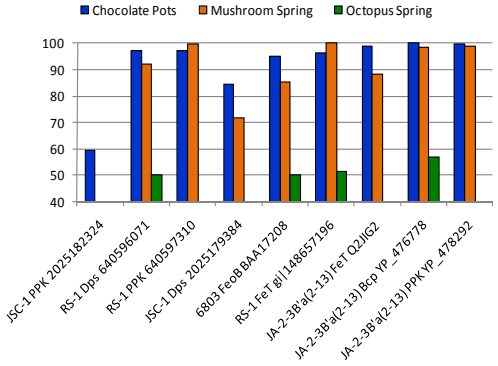
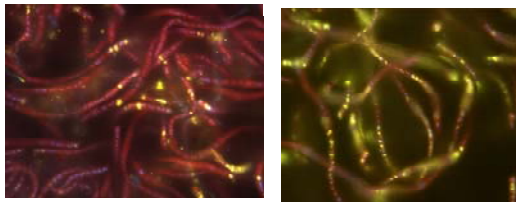


Fig. 3. The effect of Fe on the generation of polyphosphate bodies (PPB) in siderophilic CB JSC-1. *Left*: No added Fe; *Right*: 600 µM Fe.



Conclusion: We suggest that Fe-rich particles located within the siderophilic CB cells are formed biologically. These results suggest that siderophilic CB use phosphates for internal Fe sequestration and that these FeOxP can be defined as useful biosignatures. Significant differences are apparent between a set of proteins involved in the maintenance of Fe homeostasis and oxidative stress protection in siderophilic and non-siderophilic CB. Further comparative analyses of IDHS metagenomes and the genomes of siderophilic CB versus non-siderophilic ones may determine the link between physical and molecular signatures.

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